WALSH FELLOWSHIPS PROGRAMME

TEAGASC WALSH FELLOWSHIPS SEMINAR 2015

RDS, Ballsbridge, Dublin 4 | Thursday, 12 November 2015

ABSTRACTS

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AGRICULTURE AND FOOD DEVELOPMENT AUTHORITY

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The Walsh Fellowships Programme: Part of the Global Science Community

Professor Gerry Boyle, Director

This year marks the 20th anniversary of the first Walsh Fellowships seminar organised in partnership with the RDS. In the intervening period, the Walsh Fellowships Programme has grown hugely in scale and geographical spread: from less than 50 students then to over 230 today; from a budget of €0.5 million to over €5 million; from exclusive reliance on core Teagasc funding to benefiting from a range of national and international competitive funding sources; from an almost exclusively Irish-born student base to a globally representative student body; and from dependence on Irish third-level institutions to placing students in some of the world's leading universities outside of Ireland. Truly, the Walsh Fellowships Programme today has been fully integrated into the global science community.

In international terms, all forms of labour are becoming increasingly mobile and competitive – including researchers and trainee researchers. Within the EU, the commitment to open borders and integrated labour markets for researchers is evident in the evolution of the European Research Area and is underpinned by many mobility measures including the Marie Curie research training programme.

Teagasc has responded to this internationalisation of science by awarding a growing number of Walsh Fellowships to academics outside of Ireland. Currently, more than 30 Walsh Fellows are working with supervisors in universities in the UK, elsewhere in Europe, the US, Canada and New Zealand. With a view to further broadening this international dimension, Teagasc instituted short-term overseas training grants in 2012 on a competitive basis to enable Irish-based Fellows to spend short training periods in approved universities and research institutes outside of Ireland. We have also partnered with the Irish Fulbright Programme to offer opportunities to postgraduate students to spend periods of time in the US.

The purpose of all of these actions is to make sure that our PhD students have opportunities to evolve in the global research environment, to allow them to develop long-term research partnerships and to develop new skills and ideas. Such experiences help strengthen the research training of Walsh Fellows, assist them in realising career objectives and develop their scientific capabilities by learning clearly-defined methods useful for their ongoing research. These initiatives also serve to strengthen Teagasc's own links with research teams in high quality overseas institutions.

Periods spent abroad enable our PhD students to benefit from learning new techniques, attend lectures by "local" experts, be part of a unique research community which itself is international and have access to resources such as archives, collections, materials and equipment that are unique to a particular location. There is also the opportunity to experience different types of PhD structures and learning experiences. In particular, students have the opportunity to benefit from a significant component of high level taught modules and from the opportunity to gain teaching experience, which would not be possible in Teagasc where no undergraduate teaching takes place.

Teagasc will continue to strengthen this international dimension to its PhD training and will endeavour to do so in association with our Irish university partners, potentially through inter-institutional Erasmus agreements. This is vital, since the small size of our research system and its constituent institutions imply that the main intellectual community – the peer group - of most researchers necessarily lies outside Ireland. Supporting doctoral students in integrating into this global community is essential.

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Developing the commercial and strategic potential of *Ensifer*-mediated transformation (EMT) ¬– a technology platform for synthesising novel crop varieties

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While *Agrobacterium*-mediated transformation (AMT) remains the primary technology to engineer novel crop varieties, freedom-to-operate issues persist in regards to regulatory and IP hurdles. In 2012, an alternative, *Ensifer adhaerens* OV14 was identified with the ability to engineer potato[1] via *Ensifer*-mediated transformation (EMT) making EMT; a viable substitute to AMT. Since then EMT; has developed into an enabling technology for the sector but challenges exist that impede its broader commercial uptake. In response, the goal of this project was to: (i) develop an optimal environment for *E. adhaerens* OV14 that impeded *in vitro* cell aggregate formation in order to support efficient EMT (ii) expand the crop host range of EMT and (iii) investigate the impact of EMT at a crop genome level. To this end, growth conditions (pH, temperature, rpm, media composition) for *E. adhaerens* OV14 were customized to deliver non-aggregate growth to OD_{600nm} 0.8 within 48hr incubation, which also delivered significantly ($P>0.01$) improved electroporation efficiencies with *E. adhaerens* OV14. [2]By designing crop specific protocols, the utility of EMT was demonstrated with barley, oilseed rape and an alternative variety of potato with transformation efficiencies of 4%, 5% and 12% attained respectively. By sequencing the sites of integration where EMT delivered gene(s) of interest into the targeted plant genome, it was concluded that EMT has the capability to deliver ‘cleaner’ gene integration events compared to AMT. Combined, the results attained here have further demonstrated to present and future users the commercial potential of EMT.

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[1] Wendt, T., Doohan, F and Mullins, E. (2012). Production of *Phytophthora infestans*-resistant potato *(Solanum tuberosum)* utilising *Ensifer adhaerens* OV14. Transgenic Research, 21(3), 567-578.

[2] Rathore, D., Lopez-Vernaza, M., Doohan, F., O'Connell, D., Lloyd, A. and Mullins, E. (2015). Profiling antibiotic resistance and electro-transformation potential of *Ensifer adhaerens* OV14; a non-Agrobacterium species capable of efficient rates of plant transformation. FEMS Microbiology Letters, published 7th August 2015.

Conserving soil fertility by identifying critical source areas of nutrient runoff risk

Ian Thomas[1,2,] Paul Murphy[3], Oliver Shine[1], Per-Erik Mellander[1], Owen Fenton[1], Faruk Djodjic[5], Daire Ó hUallacháin[4], Paul Dunlop[2] and Phil Jordan[1,2]

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Transfers of soil nutrients such as phosphorus (P) from agricultural land to waterbodies are highest at critical source areas (CSAs), where sources coincide with high mobilisation potential and hydrologically sensitive areas (HSAs), which are at highest propensity for runoff, nutrient transport and delivery via hydrologically connected pathways. CSAs must, therefore, be accurately identified in order to target mitigation measures and best management practices to reduce associated water quality degradation and conserve soil fertility. A new CSA Index is presented which provides spatial risk-based estimates of P loss potential by integrating multi-scale datasets of source, mobilisation and transport factors within a Geographical Information System. Source and mobilisation datasets include soil test P concentrations, degree of P saturation and water soluble P. Transport risk is determined using a new HSA Index which integrates Topographic Wetness Index (TWI) and soil drainage class maps and reduces risk in areas with low hydrological connectivity where reinfiltration of overland flow or topographical impediment within a flow sink is likely. It utilises high resolution (0.25-2 m) LiDAR Digital Elevation Models (DEMs) to capture microtopographic controls on flow pathways and hydrological connectivity. Risk of particulate P mobilisation and transport is determined using the Unit Stream Power Erosion Deposition model, which integrates DEM-derived flow accumulation and Revised Universal Soil Loss Equation factors. The CSA Index was applied to four intensively monitored Irish agricultural catchments with contrasting agri-environmental conditions. Results show that the new CSA Index identifies sub-field scale CSAs and breakthrough points and delivery points along CSA pathways where P is transported between fields or delivered to the drainage network. This allows the cost-effective targeting of sub-field scale measures to conserve soil fertility and reduce P transfers. The approach is scientifically robust and could also potentially be applied to identify CSAs of other diffuse nutrients and pollutants.

Selecting nitrogen fertiliser sources for improved yield and reduced gaseous emissions in spring barley production systems

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One of the main sources of GHG emissions from spring barley in Ireland is nitrous oxide (N_2O) which is linked with the application of nitrogen (N) fertilisers. Calcium ammonium nitrate (CAN) is the dominant N fertiliser used and immediately contributes to the soil nitrate pool, increasing the risk of N loss as N_2O. Switching N fertiliser source to urea could help to manage the soil nitrate pool. However, this could also increase N loss through ammonia volatilisation. The use of inhibitor technologies in conjunction with urea could potentially manage the soil mineral N pool in order to reduce these N losses. Switching fertiliser N source could be a potential mitigation strategy for the reduction of N_2O. The objectives of this study were to assess the effect of N source on (1) N2O emissions, (2) ammonia emissions and (3) grain yield and quality. Two sites with contrasting soil types and cropping history (long-term tillage vs. short-term tillage + manure) were used to measure N_2O and ammonia emissions and grain yield and quality in 2013 and 2014. Nitrous oxide emissions were measured using the static chamber technique and ammonia emissions were measured using passive shuttles. Results show that N_2O emissions from spring barley were relatively small. There was no significant difference in cumulative emissions between CAN and urea. There was a trend for reduced N_2O emissions where the inhibitor DCD was added to urea. The urease inhibitor n-BTPT reduced ammonia emissions compared to urea. Grain yield was similar regardless of the N source used. Early results from this study indicate that the use of DCD in conjunction with urea has potential to mitigate N_2O losses from spring barley production while also maintaining yields.

Characterisation of health and immunity in dairy calves using molecular and sequencing based approaches

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Persistently high morbidity and mortality associated with bovine respiratory disease (BRD) in dairy calves results in significant economic loss for farmers in Ireland and worldwide. Infectious causes of BRD include viral, bacteriological and mycoplasmal agents with calves being more susceptible during stressful rearing periods such as weaning. Therefore, the overall objective of this PhD project was to characterise health and immunity in artificially reared dairy calves with a specific focus on the weaning period. An additional objective was to develop a novel diagnostic method for BRD-causing bacteria. These objectives were achieved by conducting a series of studies using physiological, molecular and next generation sequencing approaches with artificially reared dairy calves. In study one, haematological and molecular (gene expression) responses to gradual weaning were investigated in Holstein-Friesian and Jersey calves. RNA-Seq technology was used in a second study to further examine global changes in gene expression between Holstein-Friesian and Jersey calves in response to gradual weaning. The results of these studies demonstrated transcriptional down-regulation of immune response genes in Jersey compared with Holstein-Friesian calves during the peri-weaning, weaning and immediate post-weaning periods. An additional result was that gradual weaning is welfare-friendly as it did not induce global differential gene expression or invoke a physiological stress response in dairy calves. In a third study, a novel, culture-free, bacterial 16S rDNA gene amplicon sequencing method was developed for BRD diagnostics. Using this novel approach, the microbiomes of post-mortem lung and mediastinal lymph node tissues collected from calves confirmed with BRD were characterised. *Mycoplasma bovis* was the most abundant pathogen present in both tissue types. Bacteria which are associated with BRD, but not commonly screened, were identified. Knowledge of breed-specific immune responses and superior BRD diagnostics could enable improved health management practices better tailored towards specific disease sensitivities of particular breeds of interest.

Factors that influence bulk tank milk quality

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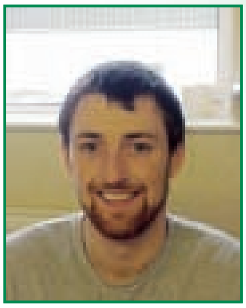
The manufacture of premium dairy products requires the production of high quality raw milk. The overall objective was to identify factors that influence the quality of bulk tank milk. Three main tasks were undertaken: the national trends in bulk tank TBC and SCC between 2007 and 2011 were assessed; the on-farm factors influencing *Bacillus cereus* in bulk tank milk were identified, and the effect of storage temperature and duration on milk quality were investigated. Monthly milk quality data was provided by 10 milk processors (suppliers=10,819). SCC increased from April onwards, with the greatest increases seen from October to December, indicating increased prevalence of subclinical mastitis in late lactation. TBC was greatest at the beginning and end of the year, coinciding with winter housing. Sixty-three farms were visited at milking time to investigate factors that influence the *B. cereus* count in bulk tank milk. Cow housing, grassland management and milking equipment cleaning were shown to influence the *B. cereus* count in milk. To measure the effect of storage temperature and duration on bulk tank milk quality, milk was stored over a 96h period at three temperatures (2°C, 4°C and 6°C) with fresh milk added at each milking. TBC and psychrotrophic count of milk increased significantly when milk was stored at 6°C, with no difference in bacterial counts when stored at 2°C and 4°C. Extended storage time negatively affected heat and rennet coagulation times and curd firmness of milk - most likely due to the dissociation of β-CN during cold storage which can be reversed upon pasteurisation. These findings suggest that good quality milk can be stored at 2°C and 4°C for up to 96 h with minimal deterioration of quality.



Understanding nutrition as a driver of flock performance

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The energy requirements of sheep are predominantly satisfied by grazed or conserved grass (90-95%), with the remainder coming from concentrate allocations during late pregnancy and early lactation. Nutrition during both late pregnancy and early lactation has been shown to have significant impacts on flock performance. This research project focused on components of nutritional management of the flock during these periods, as well as looking at how flock body condition score (BCS) throughout the production cycle impacted flock performance. A key focus was looking at the milk production ability of the ewe during early lactation. Milk production ability was assessed through either milking ewes or by analysing lamb growth rates, which are highly correlated to ewe milk production during early lactation. Based on lamb growth rates, ewe milk production was unaffected by increasing the energy content of the pre-partum diet by up to 20% where ewes were in correct BCS. Similarly, where ewes were in correct BCS at parturition and offered sufficient quantities of high quality grazed grass, the supplementation of concentrates during early lactation had no significant impact on milk production to six weeks *post-partum*. A key finding coming from this research is the importance of feeding high quality forages in late pregnancy and early lactation in order to reduce the concentrate requirements of the flock. Feeding silage with a high DMD (74%) led to higher than predicted forage dry matter intakes and a reduction in the level of concentrates required. Reducing concentrate inputs through increased quantities of forage in the diet is a key industry target and the results of this research work would show that it's an achievable objective.

Grassland biomass retrieval using optical and radar remote sensing data

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Monitoring grassland and pastures from space using imaging satellites is becoming more and more feasible due to improve spatial, temporal and spectral resolution. A literature review and a number of published studies on grassland suggest that remote sensing community and agronomists (and farmers) are agreeing to work together in order to utilize the potential of remote sensing technologies. This is now growing from research studies to the development of real time decision support systems. Consistent and regular monitoring of the world's second largest terrestrial ecosystem is not only important for grazing industry but also for the environment where grasslands are playing a very crucial role in regularization of the carbon cycle. In this study, 12 and 6 years remote sensing times data for Moorepark and Grange was used to predict the grassland biomass. We investigate the application of classical multiple linear regression and state-of-the-art machine learning algorithms for grassland biomass retrieval. This study contributes to a detailed review of application of the satellite remote sensing data for grasslands biophysical parameters retrieval, and the application of machine learning algorithms for grassland biomass retrieval using 12 years MODIS time series. We reported that dense (8-days composite) remote sensing time series along with high quality in-situ can be used to retrieve grassland biomass with high performance (R^2=0.86). In another investigation, for first-time we demonstrated that interferometrically coherent patches (grass fields) can be exploited for biophysical parameters retrieval using TerraSAR-X Staring Spotlight mode time series. The remote sensing - based model developed for Grange was modified by integrating accumulated Growing Degree-Days information and it was observed that biomass estimation accuracy increased from R^2=0.76 to R^2=0.81. This study concludes that the remote sensing technique is highly feasible and economical for large scale grassland monitoring and biophysical parameters retrieval.

Distribution of incentives and behavioural responses of Irish cattle farmers to non-linear budget constraints

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Non-pecuniary benefits are often cited as explanation for continued cattle production in a sector plagued by low profitability issues at farm level.(Key and Roberts, 2009, Vanclay, 2004, Howley et al., 2012) In this paper, we investigate the rational response of Irish cattle farmers to *Notches* in the Potential Budget Constraint. Policy conditions associated with government taxes or transfers often create discontinuities in the budget constraints of individuals. Two distinct forms of discontinuities exist; *Notches* which create a break in the budget constraint and *Kinks* which change the slope. In this paper, we investigate the response to *Notches* introduced during the coupled direct payments era 1993-2004. Farmers received an *Extensification* payment to remain below a certain stocking rate density. Above this cut-off point, farmers received no *Extensification* payment; therefore, *Notches* create a region of strictly dominated choice above the cut-off. In a frictionless world with perfect information we would not expect farmers to be operating at a stocking density which is located in an area of *strictly dominated choice,* since lowering their stocking rate would increase both income and leisure. *Notches* create strong behavioural incentives for *Bunching* directly before the strictly dominated region. Using a non-parametric methodology and Teagasc National Farm Survey (TNFS) data from 1984 -2012, we find evidence of bunching around the cut-off points but also evidence of farmers operating in sub-optimal choice areas. We then apply traditional regression methods to uncover the drivers of this sub-optimal behaviour and identify the particular optimization frictions present.

The evidence suggests that the under-responsiveness to notches is driven by misperception or inattention to subsidy incentives. This may arise because of a low degree of subsidy literacy or from the difficulty of adjusting farm practices to keep up with policy which is changing faster than the adjustment period required by farmers and farm systems.

A potential alternative to the logistic regression? - using machine learning techniques to identify Irish farmers who planted forests

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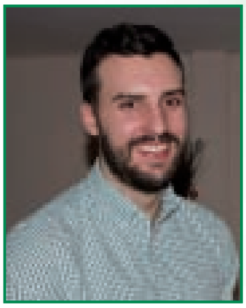
This study has two purposes. First, predictive modelling, in the form of a machine learning supervised classification, is compared with previous research that employed explanatory modelling (principal component analysis and logistic regression) to identify Irish farmers who planted forests. Both studies use the same survey dataset which provides information on 799 Irish farmers that was collected in 2011; approximately 135 questions were asked leading to over 250 potential responses. Second, an inductive data-led method identifies the group of most important responses for predicting which farmers planted forests. This contrasts to approaches taken in the extant research on afforestation which are largely deductive.

All survey responses were initially examined; recursive feature elimination with cross validation identified the group of variables that produced a model with highest predictive performance. Machine learning resulted in higher predictive accuracy than explanatory modelling and missing variables were handled with greater ease. The results show that forest owners were more likely to be inactive or retired farmers and were approached by advisory agents or staff from forestry companies to plant trees on their land. In contrast, farmers with an aversion to forest establishment display a productivist farming attitude and hold a dislike of woodlands. This research highlights the potential for machine learning methods to be used more broadly within agricultural studies.



In silico approaches for the identification of putative bacteriocin gene clusters from the human microbiota

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The gut is a rich source of antimicrobial-producers with the potential to alter intestinal communities in a beneficial way for human health. With this in mind, several studies have used traditional culture-dependent approaches to successfully identify bacteriocin-producers from the mammalian gut. Here we present alternative *in silico* techniques with the aim of detecting potential bacteriocin-encoding gene clusters using genomic and metagenomic data from the gastrointestinal (GI) subset of the Human Microbiome Project (HMP) and compare the density of these clusters to other body sites. A Profile Hidden Markov Model (PHMM) was used to search the stool and oral subsets of the HMP's Illumina Whole Genome Shotgun Assemblies database for Class I lantibiotic modification (LanB) proteins, while the web-based bacteriocin genome-mining tool BAGEL3 was used to search the GI subset of the HMP's Reference Genome Database for potential bacteriocin gene clusters. These techniques have resulted in the identification of numerous putative bacteriocin gene clusters from the human microbiota, including important members of the gastrointestinal tract microbiota that have not previously been associated with bacteriocin production. These *in silico* techniques, and others, are a powerful tool for the identification novel biosynthetic gene clusters in a culture-independent method and have the potential to vastly improve our arsenal of microbiota-modulating probiotics.

PLeASURe: Structure function-relationships of cheeses during heating and cooling

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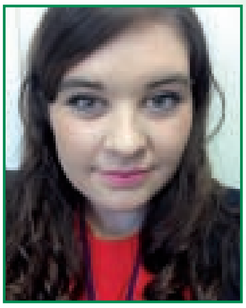
Cheese is a popular food commodity consumed as both a food in its own right and as an ingredient in many dishes, e.g. pizza. Experimental cheeses were evaluated for changes in microstructure and viscoelastic on heating from 25 to 90 °C and cooling back to 25°C to simulate the cooking (melting) and congealing of cheese. Heating resulted in extensive coalescence of fat, which became more pronounced with increasing fat content. Simultaneously, the cheese elasticity (G’) decreased and the fluidity (loss tangent, LT) increased to a maximum at 60-75 °C. The temperature required to attain a given Loss tangent, or fluidity, decreased with fat content of the cheese. On cooling, the melted cheese became more elastic and less fluid. A significant temperature hysteresis was observed for G’ and LT, with the magnitude of hysteresis depending on fat content and moisture-in–non-fat substances. The study provides insights into the design of ingredient cheese and the control of its melting and congealing during cooking and food service.



Solving the Irish *Campylobacter* problem

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Campylobacter is the leading cause of bacterial associated gastroenteritis in the EU: with on-farm prevalence in broiler batches in Ireland amongst the highest in Europe (83.1%). The objectives of this research were to: 1. Investigate the role of drinkers and feeders in flock carryover, 2. Investigate caecal growth rates of *Campylobacter:* and 3. Develop an in-house biosecurity infrastructure that would reduce the incidence of flock infection and limit spread even when *Campylobacter* accesses the flock. ISO and internationally peer reviewed culture and real time PCR methods were used in these studies, with both farm and lab trials undertaken. This research demonstrated carryover via contaminated feeders and drinkers after cleaning and the rapid growth of *Campylobacter* in the ceca. Moreover, preliminary trials on an in-house bio secure unit suggested flocks could be better protected without relying on a single line of physical defence, as is currently the case. The data generated in this study will form the scientific basis for developing improved *Campylobacter* control measures on Irish poultry farms that could make a significant contribution to solving the *Campylobacter* issue.

Human gastrointestinal endogenous proteins: peptides and health effects beyond human nutrition

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Dietary bioactive peptides are amino acid sequences released by enzymatic and/or microbial hydrolysis, which may impart a health benefit beyond basic human nutrition. However, human gastrointestinal endogenous proteins (GEP) including the mucins, serum albumin, digestive enzymes, and proteins from sloughed-off epithelial cells and bacterial cells, have only recently been considered as a source of bioactive peptides. Antioxidative GEP-derived peptides, like their dietary counterparts, may play an important role in the prevention of various gastrointestinal tract (GIT) diseases and oxidative damage. In the present work, the protein sequences of 11 GEPs, including mucin 7, serum albumin, pepsin, salivary amylase, trypsin, cholecystokinin, somato-statin-28, Ghrelin-28, lysozyme, keratin from the oral mucosa and Cas2 from *Lactobacillus rhamnosus*, were studied as a potential source of antioxidative peptides. Following simulated *in silico* GIT digestion, resultant peptides were screened against the BIOPEP database to identify unique peptide sequences. Further, the rules of quantitative structure activity relationship was applied to these unique peptide sequences and 19 novel peptides were synthesised and screened for their antioxidant activities *in vitro* using the DPPH inhibition assay. Five novel antioxidant peptides with the amino acid sequences CCK, RPCF, CRPK, QQCP and DCR were identified from the GEPs serum albumin, mucin 7 and salivary amylase. The DPPH inhibition IC^{50} values of CCK, RPCF and CRPK were found to be 28.95 ± 0.34, 497.88 ± 113.45 and 91.81± 17.64 µM, respectively. The results of this study indicate that GEPs are a significant source of antioxidative peptides. From a nutrition perspective, it is of note that secretion of GEPs is known to be affected by specific dietary components such as protein, fibre, and anti-nutritional factors. Thus, manipulation of our diet could assist in modulating GEPs secretion and therefore, the resultant bioactive peptides with physiological effects.

POSTER ABSTRACTS

Effect of tillage practices on the microbiota of winter oilseed rape under Irish agronomic conditions
R. Rathore

Investigating the soil nitrogen supply from arable soils in Ireland
S. Walsh

A Question of quality: soil carbon sequestration and storage potential of Irish grasslands
G. Torres-Sallan

Resistance to and recovery from drought conditions in multi-species grassland swards
E. Haughey

The effect of residence time on groundwater denitrification rates in two agricultural river catchments
E. McAleer

Whole-exome sequencing of Irish AI bulls with divergent fertility phenotypes
R. Whiston

Anthelmintic resistance in gastrointestinal nematodes of sheep: molecular characterisation and management
J. Keegan

Pre-weaning dairy calf management practices and their association with calf health and growth
C. Cummins

Evaluating animal and sward factors affecting *In Vivo* digestibility of perennial ryegrass
B. Garry

Strategies to increase white clover use in intensive dairy productions systems
M. Egan

Grass for biogas: optimising batch assays for defining the biomethane potential of agri-feedstocks
P. Nolan

Comparison of early- and late-maturing suckler steers slaughtered prior to a second winter
D. Marren

A novel superior gas chromatographic method for free fatty acid analysis of dairy products
D. Mannion

The fifth quarter: bovine blood as a source of functional food ingredients
T. Lafarga

Obesibiotics; identifying bacteriocin producers from the gut with a view to targeting the obesity-associated microbial populations
J. Hegarty

Glycomacropeptide from bovine milk reduces intestinal *E. coli* colonisation and associated barrier dysfunction in-vitro
S. Feeney

Targeting the glycans of bovine milk fat to reduce *E. coli* infection *in vitro*
S. Ross

Antioxidant activity-guided fractionation of major polyphenols of barley (Hordeum vulgare)
N. Gangopadhyay

Eating quality of young Holstein-Friesian bull beef is affected by feeding treatment
Y. Nian

A mixture design approach on technological and shelf-life characteristics of beef patties enhanced with clean label protein ingredients targeted at elderly consumers
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R. Reid

Effect of tillage practices on the microbiota of winter oilseed rape under Irish agronomic conditions

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Winter oilseed rape (wOSR) is an important crop in Ireland and the EU. This crop is often used as a break and rotational crop for cereals to limit fungal disease pressure. Low and reduced input systems are being investigated to reduce the costs of crop production. Changing wOSR establishment from conventional tillage to conservation tillage would be a cost effective, more efficient and eco-friendly mode of cultivation. Tillage systems influence soil physical and chemical characteristics and bring about changes in the soil microbial community structure and function. Land plants host distinct microbial communities in the vicinity of and inside their root and shoot. These communities designated rhizosphere and endosphere microbiota establish relationships with their host which range from parasitism to mutualism. However, how entire microbial communities capable of executing both growth-promoting and - compromising activities interact with the host plant, remains largely unknown. To gain novel insights on the molecular cues underlying microbial recruitment, we deployed state-of-the-art sequencing and computational approaches to characterise the microbiota retrieved from different plant compartments (bulk soil, rhizosphere, root and shoot) in two cultivation systems; conventional tillage and conservation tillage at harvesting stage of wOSR. The bacterial taxonomic diversity was analysed by Illumina Miseq next generation sequencing of V3-V4 region of the 16S rRNA gene. Genetic diversity of bacteria was higher in the conservation tillage compared to the conventional tillage, specifically in endophytic compartments. Proteobacteria were the dominant species in both tillage systems, although the phyla Proteobacteria, Firmicutes and Bacteroidetes were significantly higher in conservation root than conventional root. In summary, these studies provide a foundation for understanding the impact of cultivation practices on interactions between wOSR plants and their associated microbiota that could be helpful to develop conservation management practices of wOSR, which may provide for more sustainable crop production of wOSR and cereals.

Investigating the soil nitrogen supply from arable soils in Ireland

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Efficient nitrogen use protects our environment from pollution and also increases profit for farmers. The soil can supply considerable amounts of available nitrogen to plants. Fertiliser nitrogen recommendations should be adjusted according to the soil nitrogen supply so that excess nitrogen is not applied. This study, the first to provide data to examine the nitrogen index system in Ireland, investigated soil nitrogen supply on arable soils in Ireland as affected by cropping system, soil characteristics and weather. A total of 130 trial plots were set up over a three year experiment from 2012-2014 in winter wheat fields across 10 counties in the tillage region of Ireland. In order to examine the soil nitrogen supply these plots did not receive any fertiliser nitrogen and had different previous crops and soil types. The varying locations and years allowed different weather conditions to be examined. Soil mineral nitrogen and crop nitrogen uptake at harvest, which is a measure of the soil nitrogen supply, were the two main measurements taken. Previous crop had the most significant effect on soil nitrogen supply. Soil nitrogen supply from soils following grass was high, and following cereals was low. However, the soil nitrogen supply from soils following legumes was low and maize was high, which is not in keeping with our current nitrogen index system in Ireland. Previous crop alone is not a reliable predictor of soil nitrogen supply. Spring soil mineral nitrogen content was positively correlated with soil nitrogen supply. Soil organic matter significantly affected soil nitrogen supply, higher soil organic matter levels resulted in higher soil nitrogen supply. Soil nitrogen supply was negatively correlated with the amount of winter rainfall (Oct-Feb).

A question of quality: soil carbon sequestration and storage potential of Irish grasslands

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Soil organic carbon (SOC) is a vital component of healthy soils supporting several soil functions and comprising the largest terrestrial sink for atmospheric carbon. Soil carbon inventories and soil carbon models generally assess bulk SOC stocks to 30 cm depth, with SOC both at greater depth and in recalcitrant pools poorly studied. This study assessed the role of soil aggregate fractions across depth in order to account for the differences in SOC at thirty grassland sites covering the range of main soil types occurring in Ireland. A one kg sample was collected for each horizon to 1 m depth, sieved at 8 mm and dried at 40°C. Through a wet sieving procedure, four aggregate sizes were isolated: large macroaggregates (>2000 µm); macroaggregates (250-2000 µm); microaggregates (53-250 µm) and silt & clay (<53 µm). Organic carbon associated with each fraction was analysed. For all soil types, only the most labile carbon fractions (i.e. carbon contained in bigger aggregate sizes) are included in the top 30 cm. At depth, the Typical Surface-water Gleys and Luvisols have a bigger proportion of carbon contained in smaller fractions (i.e. enhanced carbon sequestration). Data demonstrates that Irish grasslands have a high capacity to store and permanently sequester C and provides insights to the mechanisms controlling sequestration. These results indicate (1) carbon inventories and carbon models need to account for SOC at depths greater than 30 cm; (2) fractionation of soils is necessary in order to have a better understanding of the quality of the carbon contained in soils; and (3) differences between soil types in their ability to store recalcitrant carbon at depth has implications for soil management aimed at off-setting greenhouse gas emissions.

Resistance to and recovery from drought conditions in multi-species grassland swards

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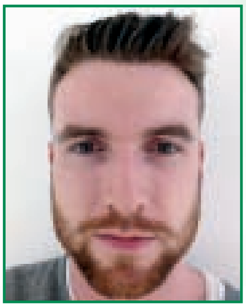
Climate change is expected to cause increased variability in precipitation patterns and a higher occurrence of drought and flooding events. Ensuring that forage production systems can cope with these challenges will require adaptation measures. There is potential to increase agricultural grassland productivity in a sustainable manner using modest increases in plant diversity. We hypothesized that four-species grassland swards would be more resistant and resilient than monoculture swards to the effects of drought. In May 2012 at Johnstown Castle, Co. Wexford, field plots (3 m x 5 m) were sown with: *Lolium perenne, Cichorium intybus, Trifolium repens* and *Trifolium pratense*. Each of the four species was sown as monoculture swards and equi-proportional four-species swards. Plots were fertilised at a rate of 130 kg ha^{-1} yr^{-1} of nitrogen and above ground biomass was removed at a height of 5 cm approximately every six weeks during the growing season from April to November. In July 2013, an experimental drought was created using 'rain-out' shelters which were placed over half of the plots. The biomass production of all communities was severely affected by the drought treatment (-84.5% on average), only the deep-rooted *C. intybus* monocultures showed some resistance (-57.4%). At the post-drought (recovery) stage, the four-species swards recovered best. Annually, four-species swards produced more biomass than the average of the four-monoculture swards (overyielding) under rain-fed (+28.4%) and droughted (+28.6%) conditions. Contrary to our hypothesis, four-species swards did not have better resistance to drought than the monocultures. However, four-species swards did have the highest recovery from drought in proportionate terms. These results highlight the complexity of individual species' responses to drought stress and importance of drought duration and intensity. There may be a role for four-species swards in mitigating drought stress through higher recovery rates and high annual biomass production.



The effect of residence time on groundwater *denitrification* rates in two agricultural river catchments

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Depending upon a complex interaction of environmental parameters, groundwater denitrification rates vary between 0 - 100%, with groundwater residence time acting as an overarching constraint. Spatial and temporal monitoring was undertaken along four hillslopes in two ca. 10km^2 catchments. Both catchments are characterised by well-drained soils, but exhibit contrasting subsurface lithologies (fractured slate vs. sandstone). The capacity for groundwater denitrification was assessed by measuring the concentration and distribution patterns of N species and aquifer hydro-geochemistry, from shallow and deep groundwater pathways, in monthly samples from a network of piezometers (*n*=37). The gaseous products of denitrification (excess N^2 and N^2O) were measured seasonally while residence times from source to stream were calculated using a two dimensional groundwater flow model (MODFLOW). The slate catchment was characterised by high permeability, which was extensive with depth. Short residence times, ranging from two months to one year in the shallow and deep groundwater pathways respectively, resulted in aerobic conditions throughout the aquifer. The five year spatio-temporal mean nitrate (NO^3-) concentration of 6.8 mg N/L exhibited limited variation throughout the sampling period (SE: 0.06, CV: 25%), with elevated dissolved oxygen across hillslope zones and depths resulting in negligible denitrification rates (typically <10%). Groundwater in the sandstone catchment had a lower mean NO_3- concentration (5.6 mg N/L) but exhibited substantially greater variability (SE: 0.12, CV: 81%). Residence times varied from six months to one year in the shallow groundwater, with travel times in excess of four years in the deeper pathways. Longer residence times facilitated the development of geochemical gradients, particularly along deeper pathways and in near stream zones. Concurrent denitrification rates in excess of 80% indicated significant NO^3- removal pathways. Results in both catchments indicated that hydrogeological setting significantly affected the distribution of groundwater NO^3-, with denitrification acting as a governing process.

Whole-exome sequencing of Irish AI bulls with divergent fertility phenotypes

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Bovine fertility has been identified as a major problem for the Irish dairy industry. Holstein-Friesian pregnancy rates can fall as low as 25%, yet no single diagnostic test can accurately predict fertility in bulls. In order to identify genetic variants affecting fertility, whole-exome sequencing of Irish AI bulls was performed using the Roche Nimblegen Developer system. Exome target design captured 202,899 exon regions (almost 57MB), including 100bp of the 5'UTR. Pregnancy rate and adjusted animal model (AAM also accounts for environmental factors including AI technician, cow health and day of the week) phenotypic records for 7,000 AI bulls were obtained; filtered and 24 bulls of high- and low-fertility were selected for sequencing. Mean target coverage of whole-exome regions was 18.5X, with 78% of the exome covered at 10X depth. GATK SNP calling identified 258,870 SNPs, 12,124 insertions and 13,048 deletions. Of these, 38% were located within exons and 2.5% were located within the 5'UTR. Of the exon mutations, 16% were non-synonymous, 17% synonymous and <1% resulted in a frameshift. SNPs with a frequency difference >25% were retained, resulting in 3,314 variants divergent between high and low fertility bulls. Furthermore, SNP association analysis identified 405 SNPs significantly associated with fertility (P<0.01). The most significant SNP was located in 3'UTR of PGRMC1 gene, the human homolog of which functions in steroid signalling, p450 activation and drug metabolism. From these SNPs discovered in bulls of divergent phenotypes, 669 have been added to the International Dairy and Beef SNP chip (v3) used for national genotyping and will determine their association with fertility in large numbers of independent samples with reliable phenotypes. Finally, specific SNPs of interest will undergo functional validation to characterise their role in bull fertility.

Anthelmintic resistance in gastrointestinal nematodes of sheep: molecular characterisation and management

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Anthelmintic resistance (AR) in gastrointestinal nematodes is an issue threatening the sustainability of sheep production worldwide. Information regarding the extent of the problem and strategies to delay the development and spread of AR are required. The extent of anthelmintic treatment failure in Irish flocks was examined by means of a drench test. More detailed investigations were carried out on four farms with a history of suspect ivermectin (IVM) resistance. Resistant nematodes were identified and isolated from two farms and their resistance profile to benzimidazole (BZ) and levamisole (LM) and Macrocyclic lactones (ML) was determined following induced infections of the respective isolates to naive lambs. The transcriptome of resistant nematodes exposed and unexposed to ivermectin was sequenced in order to determine those genes switched on in response to ivermectin exposure. Finally, a targeted selective treatment study (TST) was carried out to test whether lamb live weight was a suitable criteria to select animals who needed treatment.

Anthelmintic treatment failure was observed on 51% of flocks. The efficacy of BZ, LM and ML treatment was 30%, 54% and 74% respectively. Two farms were confirmed to have multiple drug resistant *Teladorsagia circumcincta* populations. Faecal egg count reductions of 47%, 92% and 50% for isolate 1 and 84%, 89% and 74% for isolate 2 were observed for BZ, LEV and IVM respectively. Results from the TST study showed that untreated lambs gained less weight (1.2 kg, s.e 0.20 kg) than treated lambs, independent of original weight class. As heavier lambs are likely to be sold earlier it would be appropriate to selectively treat lighter lambs. These results shed light on the current extent of AR in Ireland and emphasise the need to implement strategies to delay the development and spread of AR around the country.

Pre-weaning dairy calf management practices and their association with calf health and growth

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Calf diseases are one of the top five animal health concerns in Ireland at present. The objective of this study was to determine pre-weaning calf management practices on spring-calving Irish dairy farms, and quantify their associations with herd size, expansion, calf mortality and age at first calving. Information was available from a survey of 262 commercial dairy farms, representative of the Irish national population. Herd expansion was not associated with management practices investigated. Results indicate that colostrum management is suboptimal according to international standards. It is important that each calf receives sufficient colostrum and this can be achieved through artificially feeding it colostrum from a single cow. In this study, pooled colostrum was fed to calves by 23% of respondents; 60% fed colostrum from their own dam, 66% of which allowed the calf to suckle the dam. Larger herds were more likely to feed pooled colostrum, while smaller herds were more likely to allow suckling of the dam both of which can transmit diseases such as Johne's and cryptosporidium. Results indicate that management on larger farms particularly could be improved (e.g. rather than pooling colostrum, feed calves colostrum from a single cow). Calf mortality rate and age of first calving were lower on farms where calves were sold <6 weeks of age (49% of farms). Increased levels of hygiene (e.g. using hot water with detergent to clean feeding equipment daily) was associated with lower calf mortality and calving heifers ≤26 months of age. On specialist dairy farms excess calves should be sold earlier and greater attention to detail such as increased levels of hygiene should be implemented to reduce calf mortality and increase calf growth rates. Expanding herds are not a particular concern as expansion, independent of herd size, does not seem to alter management practices in Irish dairy herds.

Evaluating animal and sward factors affecting *in vivo* digestibility of perennial ryegrass

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Grazed grass quality is a key driver of profitability in grass-based ruminant production systems. Animal performance is dependent on the digestibility and intake of grass. The purpose of this thesis was to evaluate how animal and sward factors affect *in vivo* digestibility of perennial ryegrass (PRG). Experimental work was conducted at Teagasc AGRIC Moorepark, evaluating pre-grazing herbage mass (PGHM), grass dry matter content and PRG cultivar as sward effects, and feeding level and animal species as animal effects, on *in vivo* digestibility of PRG. The PGHM (a key grassland management tool) of grass affected *in vivo* digestibility in sheep throughout the year, with 1200 kg DM/ha maintaining greater sward digestibility throughout the year compared to 2500 kg DM/ha and 4000 kg DM/ha. High PGHM grass had a greater reduction in sward quality as the year progressed compared to low PGHM. This was due to morphological changes in the grass. No effect of PRG cultivar on *in vivo* digestibility in sheep was found. However, tetraploid cultivars maintained higher digestibility in autumn compared to diploid cultivars, reflecting the lower proportion of dead material in the tetraploid swards at this time. When investigating the effect of feeding level and dry matter content on *in vivo* digestibility in sheep, feeding at maintenance increased digestibility by 14 g/kg compared to *ad libitum* and feeding wilted grass increased digestibility by 11 g/kg compared to fresh grass. The *in vivo* digestibility of lactating dairy cows and wether sheep was compared. Differences in digestibility between the species can be attributed to an interaction between species and time of year due to changes animal digestive physiology and sward morphology. In total, eighty-nine grass samples were evaluated for *in vivo* digestibility. These grass samples will allow for future calibration of *in vitro* and NIRS techniques to predict *in vivo* digestibility. The results from this thesis provide further insight into the factors affecting PRG digestibility.

Strategies to increase white clover use in intensive dairy productions systems

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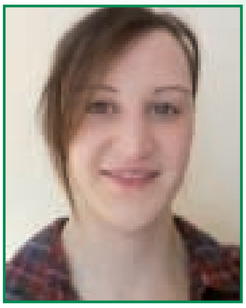
Perennial ryegrass (PRG)/white clover (clover) swards can provide a sustainable source of nitrogen (N) for intensive grazing systems and can improve herbage quality. Much of the previous research on PRG/clover swards was undertaken at low stocking rates. The objective of this thesis is to examine if clover can increase the productivity of intensive pasture-based milk production systems. In this thesis, aspects related to the effect of including clover into N fertilised PRG swards on herbage production and dairy cow milk production and rumen degradation were investigated. In grazed swards herbage production was not significantly increased with the inclusion of clover. Sward clover content in the grazing experiments was >226 g/kg DM despite the high level of N fertiliser applied (150 and 250 kg N/ha). Every 10 kg N increase in N fertiliser from 150 kg N/ha to 250 kg N/ha resulted in a decrease in sward clover content of 4.1 g/kg DM. The inclusion of clover into PRG swards resulted in an increase in milk production (up to 33 kg milk solids/cow), particularly in the second half of the year when sward clover content was >200 g/kg DM. Herbage neutral detergent fiber (NDF) was reduced when clover was included in the sward. In the first half of the year, swards (PRG only and PRG/clover) which had higher quantities of N fertiliser applied (250 kg N/ha) had greater crude protein degradability. In the second half of the year crude protein degradability was greatest on the PRG/clover swards which, at the time, had a sward clover content >290 g/kg DM. The experiments in this thesis provide evidence that the inclusion of clover into N fertilised grass swards in intensive spring-based milk production systems in Ireland can significantly increase milk solids production.



Grass for biogas: optimising batch assays for defining the biomethane potential of agri-feedstocks

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Biogas produced from the anaerobic digestion (AD) of grass biomass that is in excess of livestock requirements is one potential solution for an indigenous renewable energy supply in Ireland. Much information exists on grassland management and conservation for ruminant production systems and this may be adapted to optimising grass-biogas production system by improving the specific methane yield (SMY) of grassland feedstocks. At the laboratory-scale, sufficient processing of the biomass feedstock is required to create a representative sub-sample for analysis in the *in vitro* batch AD test. Standard method of thermal drying followed by milling does not account for losses of volatile organic compounds that are significant substrates during AD. Consequently, a novel approach of cryogenically freezing and comminuting the sample whilst frozen was applied to a diverse range of herbages and their corresponding silages, to assess the effects of preparation method on SMY, as determined in an *in vitro* batch AD test. In comparison to cryogenically freezing and milling, thermal dried milling increased ($P<0.05$) SMY of well-managed herbage and reduced ($P<0.01$) SMY of its corresponding silage (extensive lactic acid fermentation). Preparation method had no effects ($P>0.05$) on SMY of both poorly-managed herbage and its corresponding silage (extensive clostridial fermentation), or wilted herbage and its corresponding silage (restricted fermentation). Thus, thermal drying did not have a consistent effect on the SMY of the diverse feedstocks and caused a significant loss of volatile organic compounds in good quality silage (high lactic acid content). It is concluded that cryogenically freezing and subsequent milling is a more suitable preparation technique to apply than thermal drying and subsequent milling, prior to determination of specific CH4 yields, particularly for fermented feedstocks. This novel approach is now being applied to address whether applying cell-wall degrading enzymes at ensiling will improve the biomethane potential of grass silage feedstocks.

Comparison of early- and late-maturing suckler steers slaughtered prior to a second winter

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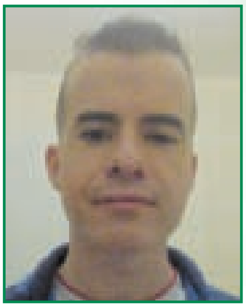
Options for finishing spring-born early- and late-maturing suckler-bred steers prior to a second winter was examined. 60 early-maturing (EM - Aberdeen Angus and Hereford sired) and 60 late-maturing (LM - Charolais and Limousin sired) weaned steers were assigned to a 2 (Breed type, EM, LM)) × 4 (Production systems, PS) factorial arrangement of treatments. All PS had a common first winter indoor period (123 days), offered grass silage *ad libitum* (GS) (DMD 688 g/kg) plus 3kg barley based concentrate daily, after which steers were assigned to their allocated PS: (1) 175 days grazing pasture only (GO), (2) 100 days GO, followed by 75 days grazing plus 4.2 kg DM concentrate daily (GGC), (3) 100 days GO, followed by 75 days *ad libitum* concentrate and GS (GAD), and (4) 175 days *ad libitum* concentrates and GS (ADLIB). Data were analysed using the Mixed Model procedure of SAS. The model contained fixed effects of breed type and PS and their interaction. Initial age was included as a covariate. Average daily gain did not differ between breed type during the winter, was greater ($P < 0.01$) for EM than LM during the first 100 d at pasture, whereas LM grew faster ($P < 0.001$) during the finishing phase. At slaughter, LM had a higher slaughter weight, kill-out proportion, carcass weight and carcass conformation score ($P<0.001$), and lower carcass fat score ($P<0.001$) than EM. Increasing concentrate inclusion in the diet increased slaughter and carcass weight, kill-out proportion and carcass conformation and fat score ($P<0.001$). On the basis that market specifications require a minimum carcass fat score of 6 (scale 1-15), only LM GO steers failed to meet the requirement. Overall, EM were lighter, fatter and had poorer carcass conformation than LM. Increasing concentrate levels in the finishing period significantly increased all carcass traits.



A novel superior gas chromatographic method for free fatty acid analysis of dairy products

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Quantification of free fatty acids (FFA) in dairy products by gas chromatography flame ionization detection is an important requirement for quality, research, nutritional, authenticity and legislative purposes. At present, procedures are typically variants of either the fatty acid methyl esterification (FAME) or direct on-column injection methods developed more than two decades ago. Both methods are time-consuming, labour intensive and environmentally unfriendly due to the significant volumes of solvents required during the lipid extraction process. This study involved the development of an alternative novel derivitisation method using butylation, incorporating automation, reducing solvent usage that is applicable for a wide range of dairy products. The accuracy, precision, linearity, limits of detection and limits of quantification of all three methods were assessed for the quantification of FFA in seventeen dairy products varying in lipid composition and degree of lipolysis. Significant limitations were evident with both the direct on-column and FAME methods. The direct on-column method experienced accumulative column phase deterioration and irreversible FFA absorption due to the acidic nature of the injection extract, which adversely impacted on method robustness and the quantification of some longer chain FFA. The FAME method experienced issues with co-elution of the injection solvent peak with butyric acid that impacted on its quantification, loss of polyunsaturated free fatty acids and the periodic emergence of artefact peaks that interfered with the quantification of some FFA. Quantification of all FFA was achieved across all samples with the novel derivitisation method. The novel butylated derivitisation step was automated, reducing solvent usage and increasing sample throughput and accuracy. Limits of quantification and detection were comparable to the other methods and the method has significant potential for research and industrial applications.

The fifth quarter: bovine blood as a source of functional food ingredients

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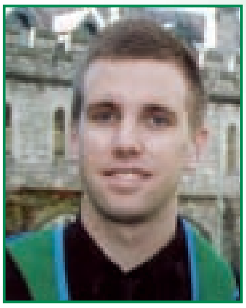
The fifth quarter refers to meat processing co-products including edible offal and blood. Rising demand for protein in countries including China and the developing nations gives the Irish beef industry an opportunity to expand its export markets. Blood is a protein-rich resource. The recovery of blood proteins and their subsequent processing into high-value bioactive peptides represents an economic opportunity for meat processors. The aim of this study was to assess the potential of bovine blood as a source of bioactive peptides that can inhibit enzymes including renin, ACE-I, and DPP-IV for use in functional foods for potential prevention of high blood pressure and diabetes type 2.

Five protein-rich fractions were generated from bovine blood by centrifugation and cold ethanol precipitation. Blood proteins were hydrolysed with papain and a number of novel bioactive peptides were identified using ultrafiltration, liquid chromatography, and mass spectrometry. Active hydrolysates were assessed for their antihypertensive potential in animal models, which are the accepted model for use prior to human intervention studies.

In this study, a number of novel bioactive peptides were identified. including the peptide SLR. which corresponds to *f*(103-105) of bovine serum albumin, and which was found to inhibit the activity of the enzymes ACE-I and renin by half at concentrations of 0.17 and 7.29 mM, respectively. In addition, the hydrolysate from which this peptide was derived was found to lower systolic blood pressure over a 24 h period after oral administration at a dose of 200 mg/Kg body weight. The identification of bioactive peptides within the sequence of bovine blood proteins would broaden the use of this resource in the food industry and may not only provide a commercial opportunity for many companies but may also have a role in improving public health.



Obesibiotics; identifying bacteriocin producers from the gut with a view to targeting the obesity-associated microbial populations

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Obesity is a complex syndrome, associated with a number of serious implications for human health including type-2 diabetes and cardiovascular disease. Obesity develops from a prolonged imbalance of energy intake and energy expenditure and while lifestyle factors, diet and exercise are key factors, the role of the microbes of the gut in obesity and associated metabolic complications has also been the focus of considerable attention in recent years. Although the specific populations involved are still the subject of debate, it has been shown, for example, that the gut microbiota of some genetically obese mice have a low abundance of the *Bacteroidetes* phylum and a high relative abundance of phylum *Firmicutes*. Other, more specific obesity-associated, microbial targets have also emerged. The intestinal microbiota is considered a rich source of antimicrobial producers, many with the ability to modulate specific components of the intestinal communities and work in our group suggested that antimicrobials can be used to manipulate the gut microbiota to treat obesity or related metabolic disorders. This study aimed to identify antimicrobials, in particular novel gut-associated bacteriocins that can be used to treat or prevent obesity. The associated methodology involved a culture-based screen for bacteriocin producers from within faecal samples from donors. More specifically, a variety of species were targeted under both aerobic and anaerobic conditions with the aim of identifying novel bacteriocin producers, followed by genome sequencing of lead isolates. A number of the bacteriocin producers that emerged from this screen are described here.

Glycomacropeptide from bovine milk reduces intestinal *E. coli* colonisation and associated barrier dysfunction *in-vitro*

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In recent years, the potential of glycosylated food components to positively influence health has received considerable attention. Milk has proven to be a rich source of biologically active glycans. Bioactivities associated with milk-derived glycans include antimicrobial, immunomodulatory, anti-adhesion, anti-inflammatory, and prebiotic. One such glycosylated component, Glycomacropeptide (GMP), can be isolated from *K-casein* after hydrolysis with chymosin during cheese making, and a number of dairy companies can produce GMP at industrial scale.

In this study, GMP was investigated for its ability to inhibit the colonisation of a variety of pathogenic *E. coli* strains to various human intestinal cell models (HT-29 and Caco-2) *in vitro*.

The protective effects of GMP against *E. coli* infection were assessed using a variety of tissue culture-based bioassays (adhesion and transwell assays). GMP significantly reduced *E. coli* colonisation, albeit with a high degree of species specificity toward enteropathogenic *Escherichia coli* (EPEC) (O125:H32 and O111:H2) ($P<0.05$) and enterohemorrhagic *Escherichia coli* (EHEC) (12900 O157:H7) ($P<0.01$). Caco-2 cells were then incubated with GMP and grown as monolayers on Transwell inserts. In the presence of GMP, *E. coli* translocation and barrier dysfunction was significantly reduced as represented by a decrease in transepithelial electrical resistance (TEER).

GMP is an effective *in vitro* inhibitor of colonisation and epithelial injury caused by *E. coli* and has potential as a bio functional ingredient in foods marketed to improve gastrointestinal health.

Targeting the glycans of bovine milk fat to reduce *E. coli* infection *in vitro*

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Escherichia coli O157:H7 is a cause of infections in humans. Severe cases of *E. coli* O157:H7 infection can lead to illnesses including haemorrhagic colitis, haemolytic uremic syndrome and even death. The emergence of antibiotic resistance means treatment of infection can be difficult thus, there is a clear need for new approaches to reduce *E. coli* O157:H7 infection. Food derived glycans are becoming an attractive alternative approach to prevent bacterial infection. These function as anti-infective compounds in the gastrointestinal tract which mimic epithelial cell surface glycans and thus act as decoy receptors which pathogens can bind to instead of the human cells. A source of these decoy glycans can be found in bovine milk fat in the form of milk fat globule membrane (MFGM). In this study, a defatted fraction of bovine MFGM (dMFGM) was prepared and the anti-infective role of this fraction against several *Escherichia coli* O157:H7 strains was demonstrated *in vitro* using an intestinal cell model in the form of HT-29 cells. We determined that this activity 1) was dependent on the concentration of the dMFGM used 2) was specific to the strain of the *E. coli* tested 3) occurred by the dMFGM interacting with the bacterial cells rather than the human cells and 4) happened immediately upon introduction of the dMFGM. These results may offer an alternative approach to alleviate the adverse health effects caused by *E. coli* infections by reducing attack of the intestinal mucosa.

Antioxidant activity-guided fractionation of major polyphenols of barley *(hordeum vulgare)*

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The role of dietary polyphenols in human health has been explored to a great extent in the past few decades. The health beneficial properties of polyphenols have been associated with their ability to reduce oxidative stress, induced by generation of free oxygen radicals in the body. Barley (*Hordeum vulgare*) is a rich source of polyphenols, and its phenolic content has been well explored in the last few decades. However, it is still unknown as to which of the individual phenolic compounds in barley are the contributing factors to its high antioxidant activity. The aim of this study was to use an approach of antioxidant activity-guided fractionation, followed by identification and quantification of the polyphenols present in the most bioactive fraction. The bound and unbound polyphenols in barley were extracted and subjected to an automated flash chromatographic fractionation, which generated 30 fractions. The obtained fractions were tested for three different antioxidant assays, the results of which correlated with each other. Fractions 4, 5, 10, 11, 15, 16 from flash chromatography demonstrated the highest antioxidant activities, fraction 4 being the most bioactive. Thus, fraction 4 was selected for determination of its polyphenolic content. Identification and quantification of the major polyphenols in this fraction was done by LC-ESI-MS/MS. The high antioxidant activity of the fraction 4 was linked to the presence of flavonols and caffeic acid in it. The flavonols that were identified were the monomer (catechin), dimer (Procyanidin B1) and the trimer (Procyanidin C1). Standards of each of the flavonols and the caffeic acid were used for making standard curves, which were then used for quantifying the respective compounds. In summary, the major contributors to the antioxidant activity of barley were determined in the above study using the approach of antioxidant activity-guided fractionation.

Eating quality of young Holstein-Friesian bull beef is affected by feeding treatment

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A high energy diet is considered to increase the tenderness, flavour intensity and juiciness of beef. However, different feeding systems show inconsistent effects on beef tenderness, cook loss and sensory attributes. Furthermore, the effects of feeding system in the first season and the level of concentrate feeding during the finishing period on beef palatability of dairy breed young bulls have not been studied. This study investigated the effects of feeding treatment (including first season) on the eating quality (tenderness, cook loss and sensory characteristics) of beef from young Holstein-Friesian (HF) bulls. Nineteen month old HF bulls were fed either 1kg or 2kg concentrates at pasture during the first season then finished either indoors on *ad libitium* concentrates or outdoors on pasture plus 5kg concentrates. The *Longissimus thoracis et lumborum* (LTL) muscle was removed from 45 cube rolls and aged for 21 days, then Warner Bratzler Shear Force (WBSF), WB slope and area, cook loss and sensory analysis were measured. Beef from bulls finished on only concentrates had lower WBSF, slope, and area, higher initial tenderness, ease of disintegration, residual roast beef flavour length and lower cohesiveness and chewiness scores than bulls finished at pasture plus concentrates. Beef from bulls fed 2kg concentrate at pasture in the first season had higher fattiness/greasiness scores during eating and after swallowing than bulls fed 1kg concentrates at pasture. There were no differences in cook loss between any feeding treatments. WBSF had a positive correlation with cohesiveness, chewiness, stringiness and a negative correlation with initial tenderness, ease of disintegration, roast beef flavor, and residual roast beef flavour length. Therefore, a higher rate of concentrate feeding during the finishing period improved both instrumental and sensory tenderness and beef flavour length. A higher rate of concentrate feeding during the first season increased the extent of sensory fattiness/greasiness.

A mixture design approach on technological and shelf life characteristics of beef patties enhanced with clean label protein ingredients targeted at elderly consumers

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High quality protein foods can play a role in reducing the risk of sarcopenia in the elderly; however, novel products must have acceptable technological and sensorial performance. Meat intake is often reduced in the elderly due to its somewhat challenging texture. Here, the technological and shelf-life properties of model meat products (beef patties) enriched in clean label protein ingredients were investigated. A screening study was conducted on lean beef patties (95% VL) formulated with pea protein isolate (PPI), rice protein (RP) and lentil flour (L) at two inclusion levels (3% and 7%) and a control. Protein content in RP7 was significantly higher than controls ($P < 0.05$). Texture profile analysis (TPA) of L enriched beef patties showed a significant ($P < 0.001$) decrease in hardness and gumminess in contrast with PPI enriched patties which did not differ significantly from controls ($P > 0.01$). Due to their acceptable properties, RP and L were then the subject of a mixture design experiment, including 17 experimental points for 3 variables (Meat 90-100%, RP 0-10%, and L 0-10%). Composition, lipid oxidation, instrumental colour, cook loss and texture parameters were assessed. L did not significantly increase protein content in beef patties; however RP increased protein content significantly ($P< 0.01$). The addition of RP with L at the higher tested proportions, increased a^* values. Models showed that increasing lentil inclusions decreased cook loss ($P < 0.0001$) and had a greater softening effect on textural parameters compared to control samples. Higher proportions of L in the formulation increased TBARS values, whereas formulations with RP only were lipid stable. Optimal beef patty formulations were defined based on the significant dependent variables (e.g. protein, TPA, colour), which will be assessed for sensory acceptability in future studies. Optimised formulations could help older adults reach targeted dietary protein requirements.

Optimisation of protein recovery from bovie lung using response surface methodology

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Bovine lung is a low value co-product of the meat processing industry, with protein content comparable to meat. A potential way to increase the value is to recover the protein for use as a functional food ingredient. The extraction of protein from lung is more challenging than protein recovery from co-products such as blood; this is in some part due to the high connective tissue content of the co- product (1). The pH shift method is a non-thermal stepwise process based on protein solubilisation using acid or alkaline conditions, followed by a targeted pH shift to precipitate the proteins and has been used with some success in the recovery of functional protein from seafood and poultry sources (2). The objective of this research was to optimise the recovery of protein from bovine lung using the pH shift process. Response surface methodology (RSM), using separate D-optimal designs was employed initially to optimise conditions for protein solubilisation followed by those for protein precipitation. Independent variables investigated for protein solubilisation were, extraction time (10-120 minutes), temperature (4-20˚C), pH (8-11) and solvent/sample ratio (2.5-10). While for protein precipitation the variables of time (0-60 minutes) and pH (4.25-6.00) were examined. The selected response of protein yield at each stage was used to evaluate the protein extraction and recovery processes. Experimental values of soluble protein yield ranged from 32.94% to 64.67% and precipitated protein yields varied from 39.94 to 66.17 %. The value for the coefficient of determination R^2 was 0.9958 for solubilisation optimisation and 0.7547 for optimisation of recovery by precipitation. The models were validated and predicted and experimental yields were found to be in good agreement. These results allowed for optimising the recovery protein from bovine lung. RSM is an effective tool to efficiently optimise protein extraction conditions.

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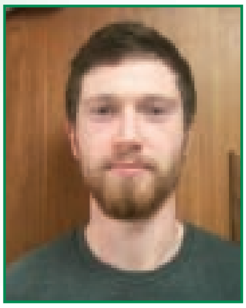

Impact of salt and salt gradients associated with brine salting on composition, microbial and metabolic activity in single and combined starter cheeses made using *S. thermophilus* & *L. helveticus*.

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This study was aimed at observing the effects of salt levels and gradients associated with brine salting on the composition and microbial activity of cheese manufactured using *S. thermophilus* and *L. helveticus* starters. Cheeses were manufactured in triplicate, brined for 66 hrs and sampled at the high-salt outside and low-salt inside layers at various time points pre-, during and post-brining. Average salt content in the outside layer post brining was ~3.8%. *S. thermophilus* counts were unaffected by salt levels whereas *L. helveticus* counts decreased significantly faster, especially in the high-salt outside layer. PepN, PepX and LDH activities were significantly lower in the outside compared to the inside layer, possibly due to high salt either thickening the cell membrane and limiting intracellular enzymes release or affecting enzymes activity. Flow cytometry (FC) analysis showed significantly reduced size and granularity of *S. thermophilus* cells in the outer layer, suggestive of cell shrinkage and condensation of internal macromolecules probably due to osmotic stress. Using FC combined with fluorescent probes assessing cytoplasmic membrane integrity and levels of free reactive oxygen species, larger membrane damage and oxidative stress was found in *L. helveticus* compared to *S. thermophilus* at all times. Also, the emergence of a new bacterial population with intact membrane and low ROS levels became evident in post-brining samples. This study showed a differential impact of salt levels on the cheese starters and highlighted cytoplasmic membrane and oxidative stresses as physiological factors contributing to the faster decline of *L. helveticus* viability.

The microbiology of beef carcasses during chilling

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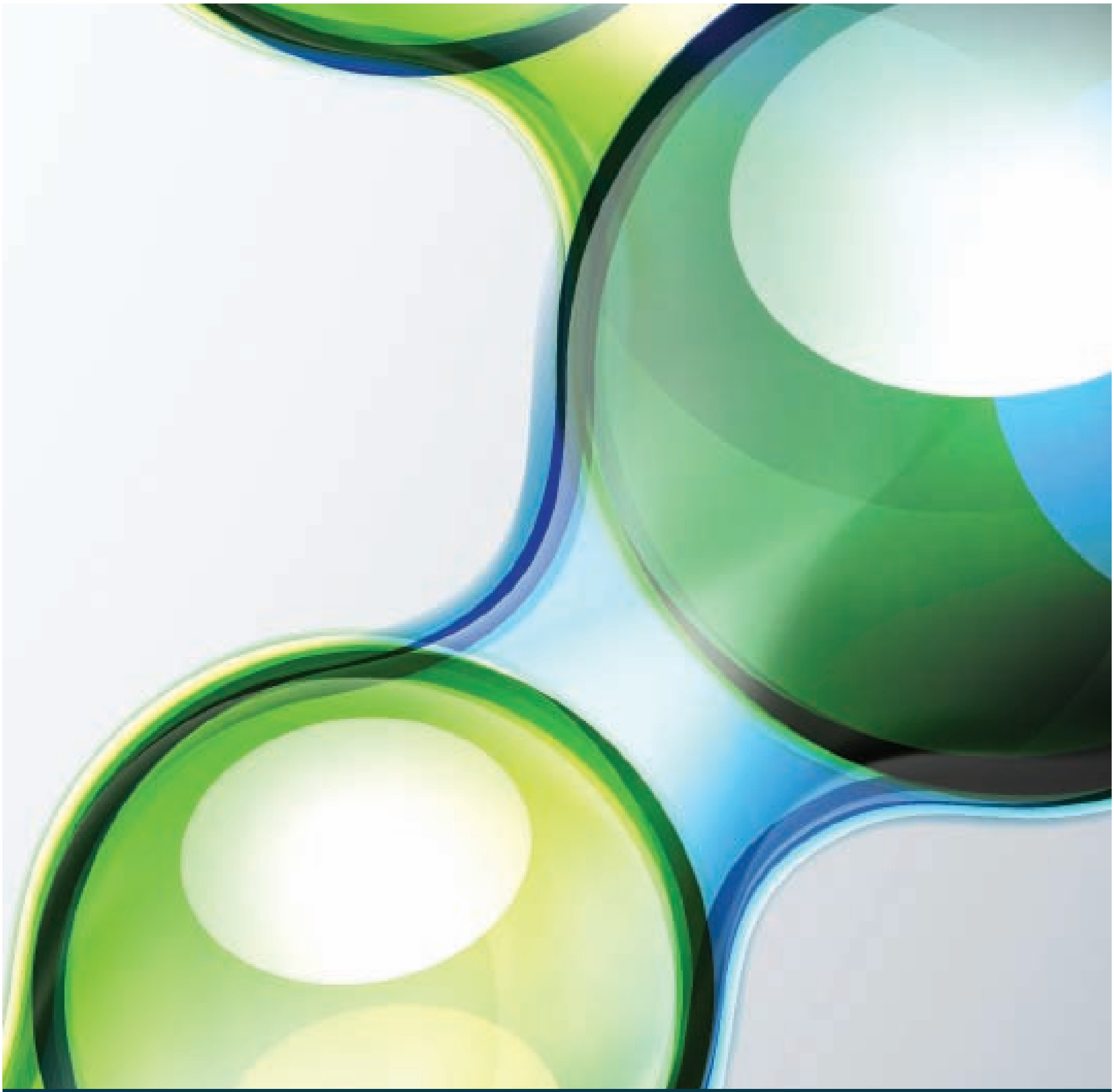
This study investigated the microbiology of beef carcasses during chilling and compared the observed data with model predictions (Combase and Food safety Spoilage Predictor) for carcasses. On three separate occasions 10 carcasses were swabbed daily at the neck, brisket, flank and rump during 4 days chilling in a commercial beef export plant and tested for TVC mesophiles, TVC psychrophiles, Total Enterobacteriacae Count (TEC), Pseudomonas spp., Clostridial spp., lactic acid bacteria (LAB) and *Brochotrix thermospachta* using ISO or internationally accepted methods. The chilling processes were also characterised in terms of ambient, carcass surface and core temperature, relative humidity, carcass surface pH and water activity (aw) for both lean and fat tissue. Carcass TVC mesophiles, TVC psychrophiles, TEC, Pseudomonas spp., Clostridial spp., and Br. thermospachta counts increased by, 0.62, 0.81, 0.51, 0.72, 0.27 and 1.42 log10 CFU/cm^2, respectively, over the 4 day chill. LAB counts decreased by 0.16 log10 CFU/cm^2. Mean pH and aw as well as the slowest and fastest chilling curves were used in the modelling studies for the carcasses. Observed growth was compared with predicted growth from models in Combase (*Br. thermosphacta* and Pseudomonas spp.) and the Food Safety & Spoilage Predictor (LAB). The study provides data on the microbiology of beef carcasses during chilling and the resultant primals after deboning. Combase accurately predicted the growth for both Pseudomonas spp. and *Br. thermosphacta* on carcasses. However, the FSSP over-estimated LAB growth on the beef carcasses during chilling. Future work in this study will include comparing the microbiology of vacuum packaged beef primals to Combase and the Food Safety Spoilage Predictor to investigate if these models can predict similar counts to the observed growth.



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